FINAL PROTOCOL

MODEL: (3LE32) Subcutaneously Implanted L1210 Leukemia

Origin of Tumor Line: Chemically induced in 1948 in the spleen and lymph nodes of a DBA mouse by painting the skin with methylcholanthrene in ethyl ether.[1]

Summary of Test Procedures: 1×10^5 cells in ascitic fluid are implanted s.c. in CD_2F_1 mice. I.P. test agent treatment begins one day after tumor implant and continues daily for a total of nine injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

ANIMALS: (refer to Protocol 8)

Propagation: DBA/2 mice (CD_2F_1 for one generation if DBA/2 are not available).

Testing: CD_2F_1 mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source for all animals in one experiment. Exceptions must be noted as comments.

EXPERIMENT SIZE: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6)

Propagation:

Tissue:

Suspension: Prepare 0.1 ml of diluted ascitic fluid containing 1×10^5 cells. Refer to Inst. 363, Protocol for Ascitic Cell Counts and Titrations.

Time: Day 6 or 7.

Site: Implant i.p. 0.1 ml of suspension.

Testing:

Tissue:

Suspension: Prepare 0.1 ml of diluted ascitic fluid containing 1×10^5 cells. Refer to Inst. 363, Protocol for Ascitic Cell Counts and Titrations.

Time: Day 6 or 7.

Site: Implant 0.1 ml of suspension s.c. in axillary region.

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1. J. National Cancer Institute, 10: pp. 179-192, 1949; and J. National Cancer Institute, 13(5): p. 1328, 1953.

INSTRUCTION 271F

WALL CHART

Model: 3LE32

Tumor: LE - L1210 Leukemia

Host:
- Propagation: DBA/2
- Testing: CD_2F_1

Approx. Tumor Transfer Day: 7
Propogation Inoculum
- Site: IP
- Tissue: 1×10^5 cells
- Level/mouse: 0.1 ml

Test Inoculum:
- Site: SC
- Tissue: 1×10^5 cells
- Level/mouse: 0.1 ml

Doubling Time: 0.4-0.6 days (acceptable range)
Approx. LE Eq.: 1.5
Mice per test: 10
Drug Route and Schedule: IP QD D1-9
Second Weigh Day: 5
Test Toxicity Day: 5

Control:
- Early Death Day: 6
- No Take Day: 18

Final Evaluation Day: 30
Parameter: Median Survival Time
Acceptable Control Range: 9-12 days

Positive Control Compound:
- NSC: 740
- Route and Treatment: IP QD D1-9
- Dose (mg/kg/inj.): 2 and 1
- T/C%: $\geq$ 135

Test T/C%:
- Toxicity: $\leq$ 85
- Grams: Negative Body Weight Change: $\geq$ 4
- Activity Criterion: $>$ 125
- DN2-criterion (if confirmed): $\geq$ 150

Model: 3LE32

TESTING SCHEDULE: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare test materials. Test positive control compound in every experiment. Record deaths daily. Refer to Protocol 10 or Inst. 361 to randomize animals. Weigh animals.

Day 1: Check cultures. Discard experiment if contaminated. Treat as instructed. Administer test agent based on average test group weights from Day 0.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Toxicity day for test animals and second animal weigh day. Prepare fresh test agent for subsequent testing based on Day 5 average test group weights.

Day 6: Control early-death day.

Day 18: Control no-take day.

Day 20: If there are no survivors except for those treated with the positive control compound, end and evaluate experiment.

Day 30: End and evaluate experiment. For special studies including schedule dependency, animals are held 60 days. Refer to Protocol 11.103 for the disposition of test survivors.

QUALITY CONTROL: (refer to Protocol 7)

Schedule the positive control compound (NSC 740* at a dose of 2 and 1 mg/kg/injection using 2% sodium bicarbonate as the vehicle) in every experiment, the regimen for which is i.p. QD 1-9. The lower T/C limit for the positive control is 135%. The acceptable untreated control median survival time is 9-12 days. Each s.c. L1210 experiment should include a titration with four inoculum levels; i.e., 1×10^6, 1×10^5 1×10^4, 1×10^3 cells (by dilution).

EVALUATION: (refer to Protocol 11)

The parameter measured is median survival time. Compute average animal body weights for Day 0 and Day 5, compute T/C for all test groups with > 65% survivors on Day 5. A T/C value of < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

CRITERIA FOR ACTIVITY:

An initial T/C $\geq$ 125% is considered necessary to demonstrate moderate activity. A reproducible T/C $\geq$ 150% is considered significant activity.

REPORTING OF DATA:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided: 1.) stating the reason for a TSC of 33, 2.) when a nonstandard dose is administered, whether due to a solubility problem or special request (Refer to Inst. 305A), and 3.) for poor suspensions.

*Positive control compound NSC 740 is Methotrexate. CAS RN is 59-05-2.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Memorandum

ate March 6, 1986

From Head, Screening Operations Section
DEB, DTP, DCT, NCI

Subject

Positive Control (NSC 740) Doses for S.C. L1210 (3LE32)

To

Screeners 03, 08, 09, 23, 28

Effective immediately, NSC 740 - the Positive Control Compound - is to be administered at doses of 4 and 3 mg/Kg/injection. This is a change from the doses listed in Instruction 367 and Instruction 271.

Betty J. Abbott

BJA:bjb

cc: Mr. Greenberg
Ms. Macdonald
Dr. Wolpert
Dr. Plowman
Mr. Johnson
Mr. Waters
Mr. Miller

Instruction 367A